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(54) Title: USE OF A SOLUTION COMPRISING GLUCOSE FOR PERITONEAL DIALYSIS HAVING REDUCED FORMATION OF AGE PRODUCTS

(57) Abstract

Use of a solution comprising glucose for peritoneal dialysis, in which the glucose portion is sterilised separately from the remaining components at a high glucose concentration above about 20 % and mixed after sterilisation, substantially according to WO 93/09820. The solution has a reduced formation of advanced glycosylation end products and the use is indicated for diabetic uremic patients.

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TITLE

USE OF A SOLUTION COMPRISING GLUCOSE FOR PERITONEAL DIALYSIS HAVING REDUCED FORMATION OF AGE PRODUCTS

AREA OF INVENTION

The present invention relates to the use of a solution comprising glucose and intended for peritoneal dialysis and having reduced formation of AGE products.

PRIOR ART

Peritoneal dialysis is a treatment performed when the renal capacity of a patient's kidney is impaired. A peritoneal dialysis solution is installed in the patients abdominal cavity via a catheter and exchange between the blood and the dialysis solution takes place across the peritoneal membrane, whereupon the dialysis solution is drained. In order to obtain fluid removal, the dialysis solution comprises an osmotic agent, usually glucose.

It is known that mixing glucose with amino-containing compounds results in a series of non-enzymatic reactions called Maillard reactions, resulting in advanced glucosylation end products, AGEs. Reference is made to WO 93/13421 and WO 95/30153. It is believed that advanced glucosylation end products are involved in the ageing process of mammals.

Advanced glycosylation end products, AGEs, are known as one candidate to cause diabetic complications. These products are derived from non-enzymatic glycosylation of long-lived proteins that are further modified by the advanced stage of the Maillard reactions, resulting in the formation of glucose-derived cross-links with a brown or fluorescent

property. These products increase the vascular permeability by several mechanisms, such as the disturbed integration of basement membrane components due to the cross-linking of proteins, the endothelial cell reaction to the AGE receptor-mediated cytokine release from macrophage, or the occupancy of endothelial cell AGE receptors.

The rise of AGEs is also associated with a variety of tissue disorders including vascular damage, dyslipidaemia and beta-2-microglobuline amyloidosis. Moreover, it has been suggested that elevated AGE levels may mediate the suppression of certain normal host defence mechanisms, such as inhibition of certain bacteriocidal activites.

Advanced glycation has also been implicated in the pathology of Alzheimer's disease.

It has also been suggested that AGE generation is enhanced by an increased oxidative stress associated with uraemia.

Formation of advanced glycosylation end products, AGEs, in connection with peritoneal dialysis has been suggested in an article "In vitro formation of advanced glycation end products in peritoneal dialysis fluid" by Edmund J. Lamb, William R. Cattell and Anne B. St. H. Dawney, published in Kidney International, Vol. 47 (1995) pages 1768-1774 (the expressions "glycosylated" and "glycated" are used interchangeably for the same property). This article investigates peritoneal dialysis solutions of conventional composition in which all components are heat sterilized together as a single solution. The article suggests that such conventional peritoneal dialysis fluid does not contain inhibitors or promoters of the early Maillard reaction other than glucose. The article states that late Maillard reaction products are formed to a higher extent in conventional peritoneal dialysis solutions compared to paired PBS controls. Moreover, the article concludes that such AGE product formation is greater in either fresh dialysis fluid or in dialysate which had been removed from patients immediately after installation,

than in dialysates which had remained for longer periods in the peritoneal cavity. The formation of protein-derived fluorescence has been used as a marker of AGE product formation. The article further concludes that results suggest either that conventional peritoneal dialysis fluid contains a factor (or factors) which promotes AGE product formation, and that its concentration diminishes during dialysis, or that the concentration of an inhibitor of the reaction increases in the dialysate during dialysis. In the article it is mentioned that glycation of peritoneal membrane proteins may be involved in the etiology of ultrafiltration failure in CAPD and should the Maillard reaction prove to be relevant to the etiology of ultrafiltration failure, it may be possible to include inhibitors of the reaction in the dialysis fluid, such as aminoguanidine.

WO 93/09820, included herein in its entirety by reference, discloses a peritoneal dialysis solution containing glucose or glucose-like compounds, such as glucose polymers. The disclosed solution is sterilised before use. During sterilisation, the glucose component is sterilized separately from the remaining components at a high concentration of glucose, over 20%, and at a low pH. After sterilisation and preferably shortly before use, the components are mixed to form the final, ready-made peritoneal solution to be installed into the abdomen of the patient. The final solution has a pH of about 6.2 - 6.5.

Separate sterilization of the concentrated glucose component as described in WO 93/09820 results in a ready-made peritoneal dialysis solution having less break-down products from glucose as compared to a conventional peritoneal dialysis solution.

DISCLOSURE OF THE INVENTION

It could be expected that all peritoneal dialysis solutions comprising glucose should induce AGE formation.

However, it has unexpectedly been discovered that the

peritoneal dialysis solution according to WO 93/09820 does not result in formation of AGE products in the same extent as conventional peritoneal dialysis solution. Consequently, a peritoneal dialysis solution according to WO 93/09820 can advantageously be used in patients where formation of AGE products is of importance, such as diabetic patients. Such use will avoid AGE related complications, such as vascular damage, dyslipidaemia and beta-2-microglobuline amyloidosis. Consequently, such use constitutes the second medical use of said peritoneal dialysis solution.

Further features and advantages of the invention will be described in more details below with reference to the appended drawings.

SHORT DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram over incubation of two PD solutions and the resultant glycation.

Fig. 2 is a diagram over incubation of the same two PD solutions as in Fig. 1 and the resultant fluorescence generation.

DETAILED DESCRIPTION OF THE INVENTION

Experiments have shown that a peritoneal dialysis (PD) solution according to WO 93/09820, in the following refered to as the PD-BIO solution, results in less formation of late Maillard products during peritoneal dialysis.

Fig. 1 is a diagram according to which a standard PD solution and a PD-BIO solution according to WO 93/09820 are compared.

The standard PD solutions had the following approximate composition in mmol/l: sodium 135, potassium 2, calcium 1,5, magnesium 0,5 and lactate 35. The final glucose concentration was 1,5%. The standard PD solution was comprised in a two litre bag and was sterilised by autoclaving the entire bag. The pH was about 5,5.

The PD-BIO solution had the same final composition.

It was comprised in a two litre bag having two separate compartments, one small, upper compartment enclosing only glucose at a concentration of about 50% and at a pH of about 3,2, and a larger, lower compartment enclosing the remaining components at a pH of about 6,7. The bag was autoclaved in this condition. Shortly before use, a frangible pin was broken whereby communication was established between the two compartments and the content of the upper compartment, glucose, was transported to the lower compartment by gravity, thereby forming the final PD solution having a pH of about 6,3.

The samples were buffered to pH 7,4 by the addition of sodium phosphate buffer to a final concentration of 50 mmol/l and was then spiked with HSA (human serum albumin) to an approximate concentration of 1 g/l.

The diagram in Fig. 1 discloses the glycation of the above-mentioned two solutions having a final glucose concentration of 1,5%. As can be seen from the diagram, there is substantially no difference in the glycation rates between the two solutions.

In Fig. 2, the same PD solutions as in Fig. 1 are shown relative to protein fluorescence generation, reflecting AGE formation according to the method described in the above-mentioned article "In vitro formation of advanced glycation end products in peritoneal dialysis fluid". As can clearly be seen, the standard PD fluid has a markedly higher generation of protein fluorescence, compared to the PD-BIO solution.

It is known that pyrraline is a marker for the presence of AGE products. As shown in table I below, the formation of pyrraline was measured for three different solutions, the PD-BIO solution, a conventional PD solution GAMBROSOL and a sterile filtered solution of the same composition as the GAMBROSOL solution, all having a glucose concentration of 4% and the same electrolyte composition as give above.

The samples were incubated for 16 hours or 7 days, at 37°C under sterile conditions with human serum albumin (A) at a

concentration of 40 g/l and without or with addition of 400 mmol/l glucose (G).

Table I

sample	incubation time	pyrraline µg/g protein	pyrraline pmol/mg protein
PD-BIO			
16-A	16 h	17	67
7-A	7 days	22	88
16-GA	16 h	19	74
7-GA	7 days	23	89
GAMBROSOL			
16-A	16 h	25	97
7-A	7 days	75	294
16-GA	16 h	24	94
7-GA	7 days	65	257
Sterile filtered			
16-A	16 h	17	66
7-A	7 days	16	65
16-GA	16 h	16	63
7-GA	7 days	22	86

As can be seen from table I, the formation of pyrraline increased by a factor 3 between the 16 hour incubation and the 7 day inhibation for the GAMBROSOL solution, but was substantially unchanged for the PD-BIO solution and the filtered GAMBROSOL solution.

Although not intended to be limited by any particular theory or hypothesis, it is believed that the above-mentioned data is indicative of the fact that conventional PD solutions sterilised in autoclaves in the final mixed composition, form degradation products of glucose acting as promoters for AGE formation. By the sterilisation method used in the PD-BIO solution, the concentration of such promoters is substantially reduced. This theory seems to be confirmed by the fact that sterile filtered PD solutions of the same composition as the

GAMBROSOL solution and the PD-BIO solution do not substantially form pyrraline, as shown in table I.

In table II there is also shown the formation of fructose lysine and pentosidine after 7 days incubation. There seems to be no difference between the three solutions in this regard. Fructose lysine and pentosidine are also markers for AGE products. The conditions were the same as for table I.

Table II

	fructose lysine		pentosidine	
sample	nmol/mg HSA	mmol/mol HSA	pmol/mg HSA	mmol/mol HSA
PD-BIO				
7-GA	79,0	5270	9,2	0,61
7-A	35,9	2390	9,5	0,63
GAMBROSOL				
7-GA	77,6	5170	10,5	0,70
7-A	31,2	2080	9,6	0,64
sterile filtered				
7-GA	73,6	4910	9,4	0,63
7-A	36,5	2430	9,1	0,61

In order to find out which components in the peritoneal dialysis solution that are responsible for the AGE formation, a series of experiments were carried out, where a sterile filtered PD solution according to the above specifications were used. To the samples of the solution were added human serum albumin at 40 mg/ml as the target protein for AGE formation. In the different samples were added glucose degradation products in different amounts typically found in heat sterilised conventional peritoneal dialysis fluids. The samples were incubated over 0, 1, 10 or 30 days and the AGE formation was measured by fluorescence measurements as in Fig. 2. The results appear from table III below.

Table III	t0	t1	t10	t30
Sterile filtered fluid	150	154	169	196
addition of acetaldehyde				
420 µmol	160	169	177	198
1573 µmol	166	173	188	218
addition of formaldehyde				
15 µmol	164	171	179	197
44 µmol	165	171	181	201
addition of methylglyoxal				
23 µmol	162	165	162	194
164 µmol	154	177	262	295
addition of 5-HMF				
30 µmol	157	156	164	193
1355 µmol	157	156	176	219
Autoclaved PD fluid	160	217	318	371

As appears from table III, there seems to be a clear correlation between autoclaved PD fluids (GAMBROSOL) and AGE product formation as indicated by fluorescence. It seems that methylglyoxal also mediates AGE product formation, while the other substances do not seem to have much influence on such production in the used concentrations.

It is known that dicarbonyl compounds and specifically 3-deoxyglycosone, 3-DG, are formed during heat sterilisation of peritoneal dialysis solutions as a glucose degradation product. 3-DG is known to be a potent cross-linker. 3-DG is a highly reactive dicarbonyl intermediate of the Maillard reactions and a precursor of the advanced glycosylation end products, AGEs, such as pyrraline. The production of 3-DG normally starts from glucose which forms a Schiffs base after reaction with the amino group of a protein.

The next step in the Maillard reactions is rearrangement to Amadori products which than in the late Maillard reactions split to form 3-DG. However, 3-DG has also been suggested as a possible intermediate in the degradation of carbohydrates by acids to 2-furaldehyde. If this is possible, 3-DG could appear in fresh fluid for peritoneal dialysis as a glucose degradation product. We have found that dicarbonyl compounds are suppressed at least tenfold in the PD-BIO solution compared to the GAMBROSOL solution.

The above-mentioned data is given for solutions intended to be used for peritoneal dialysis. When such solutions are installed into a patient undergoing peritoneal dialysis treatment, the solution comes into contact with a great number of different proteins prevailing in the peritoneal cavity. Moreover, the solution is diluted with solution already present in the abdominal cavity. Finally, an exchange of electrolytes and molecules take place inside the cavity and notably with the blood through the peritoneal membrane.

During the exposure of the peritoneal cavity to PD solutions having an unphysiologically high concentration of glucose, it is likely that proteins present in the cavity undergo similar reactions as seen in the above examples. Such altered proteins are transported from the abdominal cavity via the peritoneal membrane to the blood and to the rest of the body. Moreover, any precursors or promotors of AGE products can be transported through the periotoneal membrane into the blood.

By using a PD solution having a low concentration of promoters of late stage advanced glycosylation end products, it is likely that adverse effects on the peritoneal membrane can be avoided as well as other complications associated with the generation of AGE products.

CLAIMS

1. Use of a solution comprising glucose for peritoneal dialysis, in which the glucose portion is sterilised separately from the remaining components at a high glucose concentration above about 20% and mixed with the remaining components after sterilisation, which solution has a reduced formation of advanced glycosylation end products.

2. Use according to claim 1 in which the glucose concentration in the separate glucose portion is above about 40%.

3. Use of a solution comprising a glucose polymer for peritoneal dialysis, in which the glucose polymer portion is sterilised separately from the remaining components at a high glucose polymer concentration above about 20% and mixed after sterilisation, for reduced formation of advanced glycosylation end products.

4. Use according to claim 3 in which the glucose polymer concentration in the separate glucose polymer portion is above about 40%.

5. Use of a solution comprising glucose for peritoneal dialysis, in which the glucose portion is filter sterilised and mixed with the remaining components after sterilisation, which solution has a reduced formation of advanced glycosylation end products.

6. Use of a glucose solution, which is sterilised substantially separately at a high glucose concentration above about 20%, for providing a peritoneal dialysis solution having reduced formation of advanced glycosylation end products for treatment of AGE related deceases.

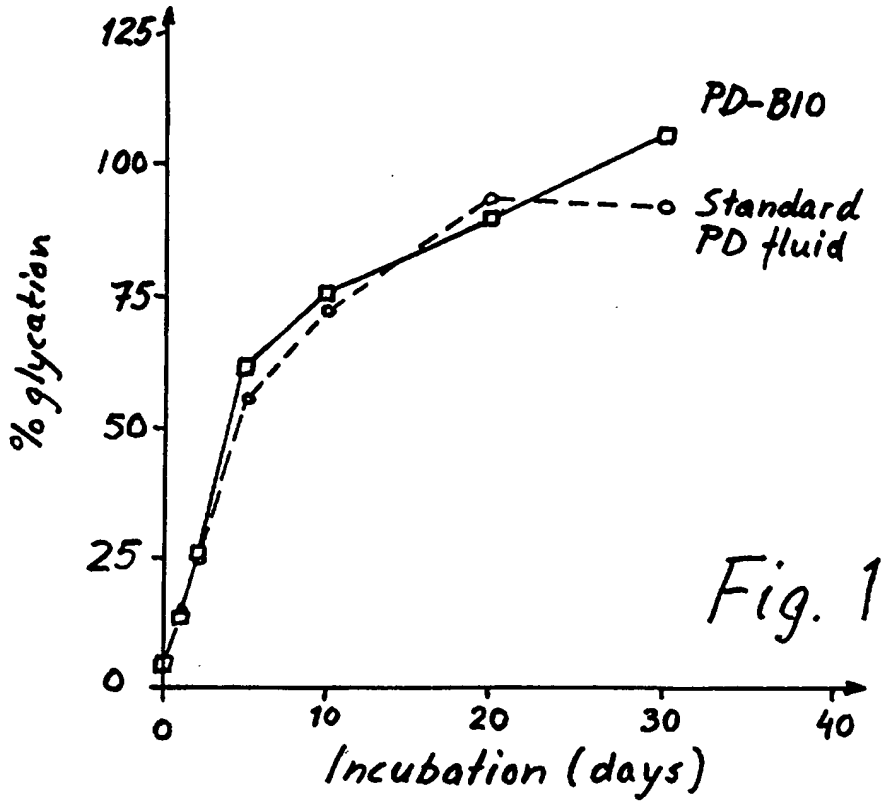
125
100
75
50
25
0
% glycation
PD-BIO
Standard
PD fluid
0
10
20
30
40
Incubation (days)

Fig. 1

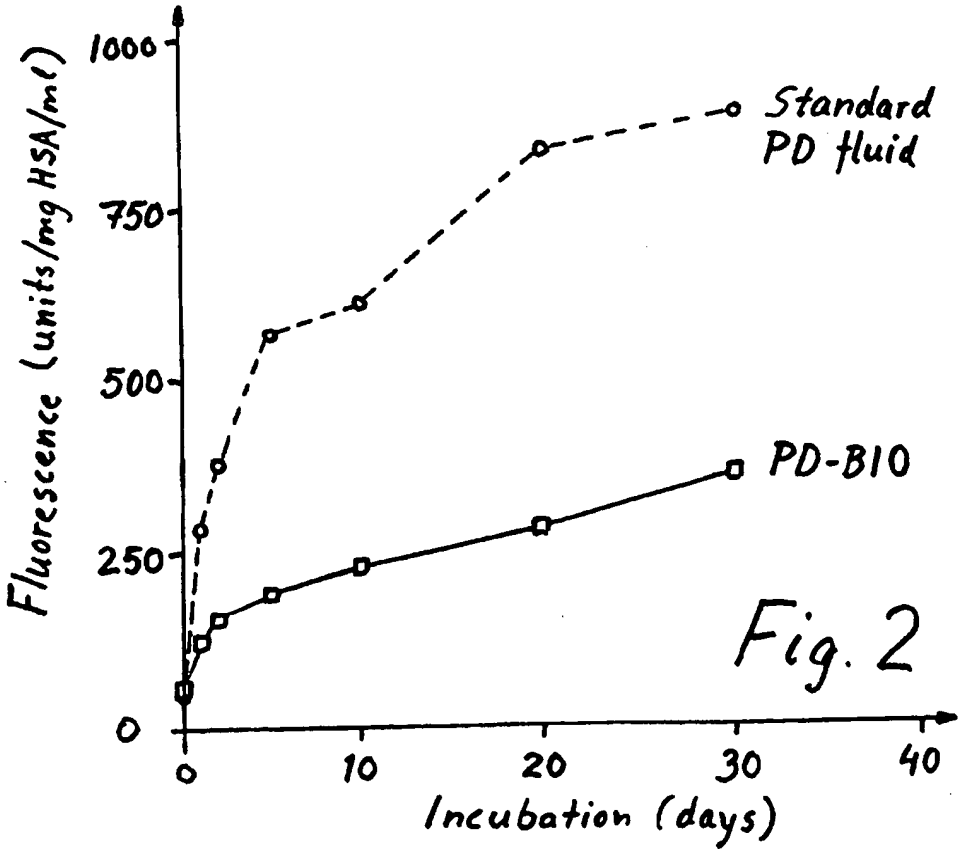
1000
750
500
250
0
Fluorescence (units/mg HSA/ml)
Standard
PD fluid
PD-BIO
0
10
20
30
40
Incubation (days)

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00272

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 9/08, A61M 1/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EMBASE, WPI, WPIL, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9309820 A1 (GAMBRO AB), 27 May 1993 (27.05.93), page 2, line 12 - line 28; page 7, line 20 - line 30; page 8, line 16 - line 21	1-6

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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 1997	18 -06- 1997
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Anneli Jönsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00272

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 6
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Claim 6 are directed to method of treatment of the human or animal body by therapy method practised on the human or animal body (Rule 39.1(iv)). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/06/97

International application No.
PCT/SE 97/00272

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9309820 A1	27/05/93	EP 0668785 A	30/08/95
		JP 7500992 T	02/02/95
		SE 9103395 D	00/00/00
		US 5536469 A	16/07/96